LYMNÆA COLUMELLA, AND SELF-FERTILIZATION.

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The following paper is a preliminary study of the pond snail, *Lymnœa columella* Say, with the particular view of its furnishing material for the study of genetics. We should look to this form because of the probability that when isolated from one another, the eggs that are laid are self-fertilized. No studies have yet been made on animals that reproduce by self-fertilization, so that, as pointed out by Jennings, 1911 B, no work on animals can be directly compared with that on plants.

Notwithstanding the many means by which hermaphroditic animals prevent self-fertilization, a number of cases are well known where normally self-fertilization does occur. As an example of this the following groups may be quoted: Rhabdocoel Turbellarians, Sekera 1906; Polystomum, Zeller 1876;[1] the digenetic Trematodes, Leucart, v. Siebold, Zaddock, Voeltzkow, and others; the Cestodes, Loess, Carlisle, Schultze, Bellingham, van Beneden, Pagenstecher, Leuckhart,[2] and others; the Ascidians *Cynthia* and *Molgula,* Morgan 1904; *Botryllus,* Pizon 1893; and the pond snail *Lymnœa.*

This paper will not discuss the adaptability of the material from the Flat Worms or the Ascidians for breeding experiments. Parasites and marine animals furnish great difficulties at the outset for such a study, and Rhabdocoels reproduce also asexually, so we will turn at once and consider the case of the pond snail.

The first point to be determined is: Does *Lymnœa* self-fertilize its eggs? Oken (1817)[3] isolated an individual adult *Lymnœa.* After an interval of some months this snail laid fertile eggs. Oken concluded that this was a case of self-fertilization. Von Ihring (1876) showed how in many gastropods, *Helix,* for example, a long period, as much as a year, may intervene between copulation and egg-laying. Because of this he did not consider Oken's experiment of any value.

[1] See Bronn, 1899 and 1900.
[2] See Bronn, 1899 and 1900.
[3] See Braun, 1888.

In the meantime von Baer (1835) reported an observation which seemed to confirm Oken's view. He actually saw *Lymnæa auricularia* with own penis inserted in its own female opening. Braun (1888) isolated eggs of *Lymnæa auricularia* in separate vessels and raised the young snails which when they reached adult size laid eggs which developed. Although this author had the snails under observation for weeks and months at a time, he never was able to confirm von Baer. Nevertheless, he did not doubt that von Baer was correct in what he saw. The present writer has repeatedly isolated eggs and had them develop into snails which laid fertile eggs, but has never witnessed a case of self-copulation.

There is the alternative that must be disposed of: Can it be that these eggs are not self-fertilized, but that they develop parthenogenetically? This question cannot as yet be fully answered. To be sure, normal parthenogenesis is unknown in mollusks, but direct evidence on the case in *Lymnæa* is lacking. The reduced number of chromosomes in maturation of the sperm is small, six to eight, but as yet the writer has been unable to satisfactorily imbed the eggs so that they may be cut. As yet he has been unable to observe the first cleavage figures.

An examination of the reproductive organs of the snail will show that there is no reason why self-fertilization should not occur. This system is a complicated one in the lung-bearing mollusks, and *Lymnæa* is no exception to the rule. The eggs and sperm arise side by side in a common ovitestis. When ripe both eggs and sperm pass down a common hermaphrodite duct. Into this duct the albumen gland opens, the function of which is to secrete around the egg a thick coat of albumen. This is the substance which makes the eggs so difficult to imbed. Past this point the duct divides into a thread-like vas deferens with wider portion called the prostate gland, and a thicker-walled oviduct. The former leads finally to an invertible penis just back of the tentacle on the right side of the animal, while the oviduct opens somewhat to the exterior somewhat posterior to the former with a narrow slit. Into the oviduct near its aperture opens the duct from the so-called sperm receptacle, in which the writer has never found sperm, but in which very often he has found eggs. He hopes to deal with this matter at another time.

The reason for describing in some detail the reproductive organs of this animal is to make clear that there is every piece of mechanism present to allow self-fertilization. If the eggs are not fertilized in the upper part of the oviduct, they may be by self-copulation in the lower part.

The fact that self-copulation had been observed by no other observer than the great von Baer, the fact that a case of parthenogenesis is as yet unreported in the group of the mollusks, together with the fact that there is every arrangement present in the animal to make self-fertilization possible—all these seem to the writer sufficient evidence that the chance that parthenogenesis plays a part is remote. This must not be taken as precluding this remote possibility.

Are these animals easy to handle in a breeding experiment? This question may be answered, on the whole, yes.

In 1908 the writer (Colton, 1908) published the results of a series of experiments on the pond snail *Lymnæa columella,* showing the effects of various external conditions upon the growth. Among other things, the writer found how easy it is to raise *Lymnæa* from the egg under ordinary laboratory conditions. They will live in as little as 500 cc. of water and require next to no care. Philadelphia city water from the tap was found to be fatal to the young snails, but the same water, after standing in a large aquarium for some time, could then be used. In his experiments this winter even this water proved fatal, so that water had to be procured from the nearby pond in the Botanical Garden of the University. In the previous experiments a water plant, *Myriophyllum,* and a little soil seemed to offer the optimum conditions for growth, aeration being neglected. This winter the writer has had the best results in using dead leaves of trees from the bottom of the pond referred to above. These were washed in running tap water as a precaution against introducing young snails from the pond. The best results are now procured by using about 700–1000 cc. of pond water in a battery jar and placing in this a half-decayed leaf, such as a maple leaf. One snail only is of course placed in the jar.

The length of time that it takes from hatching to egg-laying varies greatly. As the cause of this variation is not understood at all, it will be at the present time worth while to mention only some of the cases. Thirteen had the following interval from hatching to egg-laying: 32, 26, 35, 49, 58, 92, 50, 50, 56, 57, 63, 74, and 74 days. Twenty-six days was the shortest interval. This shows, even if we neglect the shortest periods, how surprisingly rapid is the growth. As for ease of handling in a laboratory, *Lymnæa columella* is seen to furnish splendid material. They require little care, little space, and come to maturity promptly.

What are the characters that distinguish the various species and

varieties of *Lymnæa* from one another, and what is the character of the variation within one species?

The most recent work on the systematic relationships of the various species and varieties of *Lymnæa* is the monograph on the group by F. C. Baker (1911). This writer recognizes one hundred and two recent species and varieties which he distributes through six genera as follows: *Lymnæa*, two species and three varieties; *Radix*, one species; *Bulimnæa*, one species; *Acella*, one species; *Galba*, fifty-eight species and twenty-eight varieties. The characters on which these genera are distinguished from one another are as follows: the relative size of portions of the male reproductive organs, whether there are one or two penis sac retractors; whether there are two or three cusps on the lateral teeth; proportions of the jaws, length of the spire, axis of the shell gyrate or not, sculpture of the surface, etc. It will be noticed that all these characters, with the exception of two, are purely quantitative. The character of the teeth is a qualitative difference to which the author attributed little importance, as his *Galba obrussa* has the same type of radula as his *Pseudosuccinea columella* when the latter is half-grown. There is no qualitative difference between the radulæ of the adults. Many *Galba* have tri- and other *Galba* have bi-cuspid lateral teeth. Of the one hundred and two species and varieties, of but thirty-two did the writer know aught of the anatomy of the reproductive organs. Nothing is apparently known of the internal anatomy of the type species of the genus *Galba*, that is, *Galba truncatula* Muller. Since the important characters in describing the genera are purely quantitative, the present writer feels that he cannot accept these genera and will for the present consider the old genus *Lymnæa* as including them all. When we know more, some genera may, perhaps, be farther separated. Not being a systematist, the author cannot criticise this work and he is very thankful that someone has taken the trouble to examine all the literature and the species of this interesting group, bringing it together in one work.

The individual species are separated one from the other by quantitative differences in the shell characters, body characters, by color, and where known the genital organs, the radula, and jaw. This roughly outlines the sort of variation that takes place within the genus. Within a single species what can we look for?

In the present case, where selection is hoped to be practiced, characters visible on the exterior can alone be considered. This forbids us at the outset dealing with any characters of the internal

organs. In the snail the shell at once presents itself. So far we have considered the shell alone.

Adams, 1900, made a careful study of the fresh-water prosobranch mollusk *Io* taken from many stations on the same watershed. He measured the height of the spine, the length of the aperture and width of the shell. In the present study three characters were also measured. These were chosen so that when their ratios were plotted a picture of the average shape of the shells of the colony from which the collections were made would result.

The length of the spire of the shell may be expressed by the ratio—altitude to length of aperture. See fig. 1, where $\frac{AB}{BC}$ this ratio. The width of the shell may be expressed roughly in terms of a ratio, length of the aperture to the width. The former ratio $\frac{AB}{BC}$ we will refer to as the *ratio*, and the latter ratio we will refer to as the *index*.

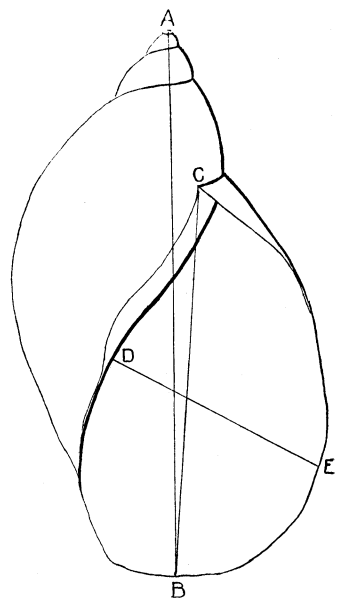

Fig. 1.

Using the measurements furnished by Baker, 1911, which are probably measurements of extreme individuals, calculating their *index* and *ratio* and plotting them, using the *index* as an ordinate and the *ratio* as the abscissa, when these are enclosed by a line, then we have a rough picture of the shape of the shell. Fig. 2 shows such a picture and the dark oval represents *Lymnœa columella* as it occurs about Philadelphia as compared with some forms taken from Baker's measurements.

The writer made a number of collections of *Lymnœa columella* from some ponds and streams about Philadelphia, and also examined several series in the collection of the Academy of Natural Sciences of Philadelphia.[4] The three characters referred to above were measured with proportional dividers, the index and ratio were calculated and plotted in groups. Fig. 3 shows a number of these collections. The

[4] The writer wishes to thank Dr. H. A. Pilsbry, of the Academy of Natural Sciences, for many courtesies.

probable error of the single *ratio* and *index* was calculated. These were based on twenty measurements of the three characters from a single shell.[5] The result of this was a probable error of ±.04 for

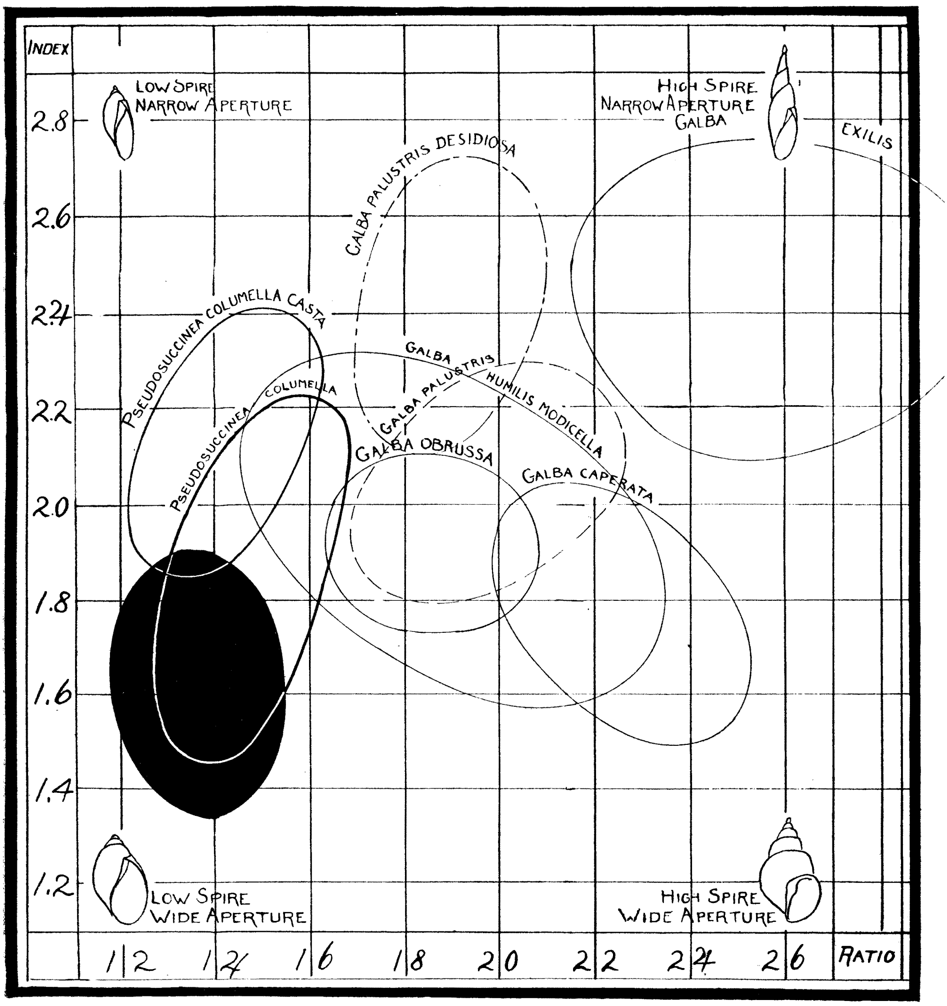

Fig. 2.

the *index* and ±.01 for the *ratio*. The probable error of the *index* was greater as this character was more difficult to measure. H. B.

[5] See Mellor (1905), pp. 515–520.

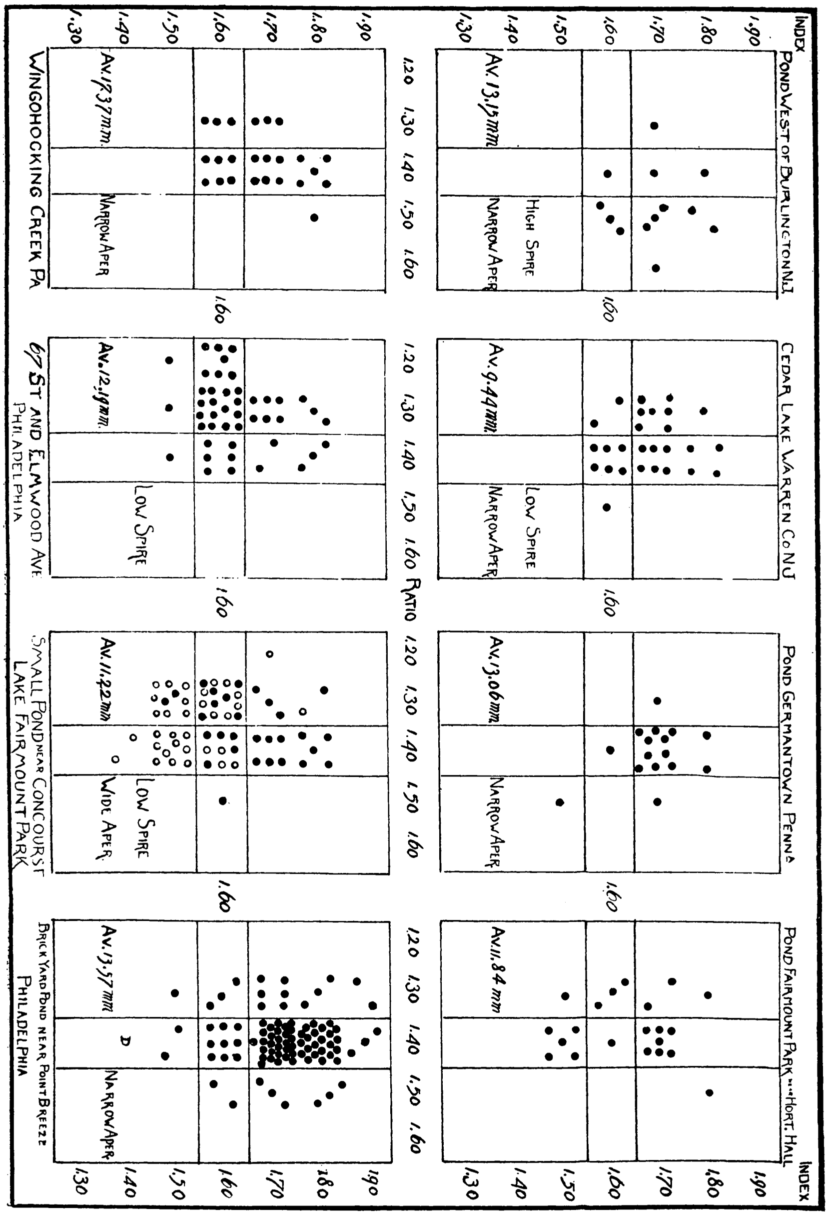
Index
Pond West of Burlington N.J.
Av. 13.15 mm
High Spire
Narrow Aper
Cedar Lake Warren Co N.J
Av. 9.44 mm
Low Spire
Narrow Aper
Pond Germantown Penna
Av. 13.06 mm
Narrow Aper
Pond Fairmount Park... Hort. Hall
Av. 11.84 mm
Index
Wingohocking Creek Pa
Av. 17.37 mm
Narrow Aper
67 St and Elmwood Ave Philadelphia
Av. 12.19 mm
Low Spire
Small Pond near Concourse Lake Fairmount Park
Av. 11.22 mm
Low Spire
Wide Aper.
Brick Yard Pond near Point Breeze Philadelphia
Av. 13.57 mm
Narrow Aper
Ratio
1.20 1.30 1.40 1.50 1.60
1.30 1.40 1.50 1.60 1.70 1.80 1.90

Fig. 3.

Baker (1910) found in *Lymnæa reflexa* that the length-breadth ratio of the shell increased with the length of the shell. That is, the larger shell had the higher ratio and therefore a higher spire. Tables I and II show this relationship in *Lymnæa columella* from about Philadelphia and on both tables the length in millimeters is the

TABLE I

INDEX	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22 mm.	TOTAL 345
1.90							1	2	2			7
1.80					4	12	16	18	2	3		55
1.70			1	2	14	16	40	25	11	4	4	117
1.60		1	3	5	17	29	26	14	4	4	1	104
1.50		2	1		7	16	12	3				21
1.40		1				2						3
MEANS		1.500	1.600	1.629	1.633	1.627	1.664	1.709	1721	1.691	1.680	1.654

TABLE II

RATIO	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22 mm.	TOTAL 345
1.60							1					1
1.50		3	1			2	8	6	2			22
1.40		1	4	3	23	39	55	34	7	6	4	176
1.30				4	16	30	26	21	10	5	1	113
1.20					3	4	5	1				13
MEANS		1.475	1.420	1.343	1.348	1.352	1.373	1.373	1358	1.345	1.380	1.362

ordinate. The *index* on Table I is the abscissa. On Table II the *ratio* is the abscissa. Table I shows that the larger shells have a narrower aperture, which agrees with H. B. Baker, but Table II shows that the height of the spire is about constant at all ages. On both tables no conclusions can be based on shells under eight millimeters, for two reasons: the numbers of shells are too small and the probable error of each ratio is too large. It may be as much as ±.10 on the part of the *index* and ±.05 for the *ratio*.

Remembering, then, that the larger shell may have a narrower aperture, then we may look over fig. 3. In this the Cedar Lake collection and the Wingohocking Creek collection both have narrow apertures, but the former has an average shell of 9.44 mm., while the latter has an average shell length of 17.37. The size of the shell cannot influence, then, the characters in question in this case. The other diagrams tell their own story and seem to show that each restricted area has its own type of shell as far as these two characters are concerned. The numbers are far too small on which to base many conclusions.

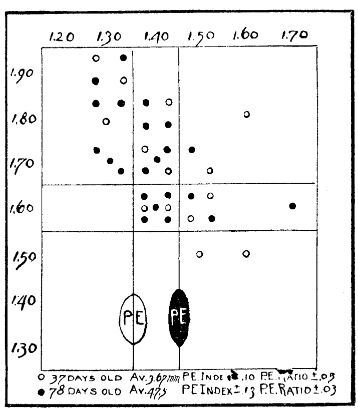

Fig. 4.

There were hatched during the past winter from an egg capsule laid by a snail from the Sixty-seventh Street and Elmwood Avenue collection thirty-two snails, of which eighteen lived to be measured. These were kept together in 1,000 cc. of water in a crystallizing dish. They were measured from time to time. From two of these measurements, an early one and a late one, 37 days and 78 days, respectively, fig. 4 was compiled.

The probable error of the individual 37 days old measurements,

which were made with the camera lucida, were *index* =.10 *ratio* ±.05. These were so much larger in the case of the small shells because of the difficulty in orienting them in exactly the same plane at the time of measurement.

The range of the offspring are almost the same as that of the parent colony. This is only significant as showing what we may expect when we are able to deal with larger numbers in a more precise manner.

Baker (1911) observed copulation between different individuals of *Lymnæa stagnalis, Lymnæa emarginata* and *Lymnæa lanceata.* Several times *L. emarginata* tried to copulate with *L. lanceata,* a much smaller animal. Baker saw also *L. stagnalis* in copulation with *emarginata.* Heynemann (1869) records a cross between *auricularia* and *peregra,* the former acting as female and the latter as male. Chaster (1909) records a cross between *L. stagnalis* and *L. auricularia,* the former taking the part of the male. The progeny were good examples of *peregra,* and the query is raised by Chaster as to whether *peregra* may not be the ancestral form of *auricularia* and *stagnalis.* Be that as it may, it is possible that many of the so-called species and varieties of *Lymnæa* may be hybrids, and it will be necessary to experiment in order to determine how true this is. In the ponds about Philadelphia, however, where *Lymnæa columella* is found, the writer has never found any other species present. The chance of hybridization is, therefore, rare.

Conclusion.

Johannsen (1911) clearly defined the "pure line." "A pure line," he said, "may be defined as the descendants from one single homozygotic organism exclusively propagating by self-fertilization. . . . A line ceases to be 'pure' when hybridization (or even intercrossing) disturbs the continuity of the self-fertilization." With this definition in mind it is necessary, if we would have a pure line in the Johannsenian sense, to deal with hermaphroditic organisms. Not only must the organisms be hermaphroditic, but self-fertilization must be possible. It must either be normal or can be induced and controlled. These conditions can easily be fulfilled in many plants, but, as far as I am aware, no animals with the proper requirements have been experimented upon.

To be sure, Jennings (1911) and others have traced "lines" of Protozoa. These animals divide by fission so that their method of reproduction is probably closely allied to asexual or to parthenogenetic reproduction as found in multicellular forms.

The *Hydra* with which Handel (1907) worked reproduced by

budding. On the other hand, Woltereck's (1909) *Daphnia* were parthenogenetic. These "lines" of animals therefore cannot be compared strictly with those "lines" in plants that reproduce through self-fertilization.

Adhering strictly to Johannsen's definition, as far as I know no experiments with *pure lines* have ever been performed with animals.

Lymnæa, after this superficial study, would seem to furnish such material:

1. It apparently does self-fertilize its eggs when isolated.
2. The time for generation is short—two to three months.
3. There are a few well-defined characters that may be observed.
4. Hybridization is possible, but as far as *Lymnæa columella* from this region is concerned, it is rarely that more than one species is found in a single habitat. There is no evidence, as yet, that it is not homozygous.

On the whole, *Lymnæa columella* seems to combine some of the necessary requirements on which to base a pure-line investigation.

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